Clinical translation of advanced colonic drug delivery technologies

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A B S T R A C T

Targeted drug delivery to the colon offers a myriad of benefits, including treatment of local diseases, direct access to unique therapeutic targets and the potential for increasing systemic drug bioavailability and efficacy. Although a range of traditional colonic delivery technologies are available, these systems exhibit inconsistent drug release due to physiological variability between and within individuals, which may be further exacerbated by underlying disease states. In recent years, significant translational and commercial advances have been made with the introduction of new technologies that incorporate independent multi-stimuli release mechanisms (pH and/or microbiota-dependent release). Harnessing these advanced technologies offers new possibilities for drug delivery via the colon, including the delivery of biopharmaceuticals, vaccines, nutrients, and microbiome therapeutics for the treatment of both local and systemic diseases. This review details the latest advances in colonic drug delivery, with an emphasis on emerging therapeutic opportunities and clinical technology translation.

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Contents

1. Introduction

The colonic region of the gastrointestinal (GI) tract provides a multitude of therapeutic opportunities. Research in this area has been driven by the need to better treat local disorders of the colon such as inflammatory bowel disease (IBD) (ulcerative colitis [UC] and Crohn's disease [CD]), irritable bowel syndrome and carcinoma [1-4]. In recent years, advances in colonic drug delivery have pivoted from not only the treatment of local diseases, but also to systemic conditions by virtue of the gut microbiome. Described as the "last organ", the human microbiome encompasses trillions of microorganisms residing within a myriad of ecological niches of the human body. The microbiome regularly shows a large degree of interpersonal diversity, even in the absence of disease [5]. The realisation that imbalances in the microbiome can influence the onset of both local and systemic diseases, however, has altered the concept of a pharmaceutical-microbiome relationship [6-8]. The microbiome now represents an intermediate, capable of metabolising and altering drug pharmacokinetics to consequently enhance or inhibit clinical response for the treatment of systemic diseases such as human immunodeficiency virus (HIV), Parkinson's disease, celiac disease and diabetes mellitus to name a few [9-15].

Additional interest in targeting the colon has stemmed from the potential of this region as a site for the entry of drugs into the systemic circulation. For example, the colon has comparably lower levels of several luminal and mucosal metabolic enzymes and transporters, offering multiple therapeutic advantages. For example, simvastatin (a CYP3A4 substrate) has been shown to have super oral bioavailability (three-times greater) when delivered to the distal gut by a delayed-release formulation when compared with an immediate-release formulation [16]. The lower proteolytic activity in the colon may also be beneficial for the delivery of biologics such as proteins, peptides and monoclonal antibodies [17-19]. Fig. 1 provides a summary of the local and systemic opportunities available for drug delivery to the colon.

Interest in colonic targeting has risen since the introduction of the first colonic prodrug, sulfasalazine, for treatment of rheumatoid arthritis and later, IBD. This drug is comprised of a 5-aminosalicylic acid (5-ASA) moiety linked to a carrier molecule, sulfapyridine, by an azo bond [20,21]. Following ingestion of the dosage form, the drug reaches the colon and the azo bond is cleaved by colonic bacteria, releasing the active 5-ASA to the local site of inflammation. Sulfasalazine has been demonstrated as effec-

tive in achieving complete remission in the majority of patients with mild to moderate UC. However, up to 50% of patients experience allergic reactions or adverse effects with therapeutic doses [22], related to the sulfapyridine moiety [23]. In attempts to avoid such unfavourable events, other prodrugs were developed and approved. Examples include olsalazine, where 5-ASA is azo bonded to another 5-ASA molecule [24]; and balsalazide in which 5-ASA is azo bonded to an inert carrier (4-aminobenzoyl-beta-alanaine) [25]. The focus was then placed on developing new formulations rather than new drugs [26]. In this regard, a number of successful modified release formulations of 5-ASA were introduced and marketed as first-line treatments for IBD (Fig. 2) [27,28].

Due to the physiological complexities of the colon, site-specific drug delivery technologies must be developed to ensure reliable delivery across patient populations [29]. Upon the turn of the millennium, colonic drug delivery strategies transitioned from traditional methods to more advanced modalities. This transition evolved from the use of a single drug delivery stimulus towards a combination of stimuli, with the ultimate goal of achieving
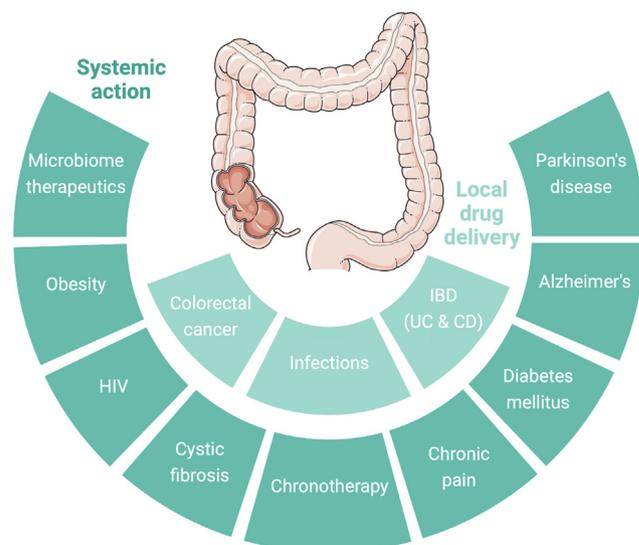
Fig. 1. A summary of the local and systemic opportunities available for drug delivery to the colon. *IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; HIV, human immunodeficiency virus.*
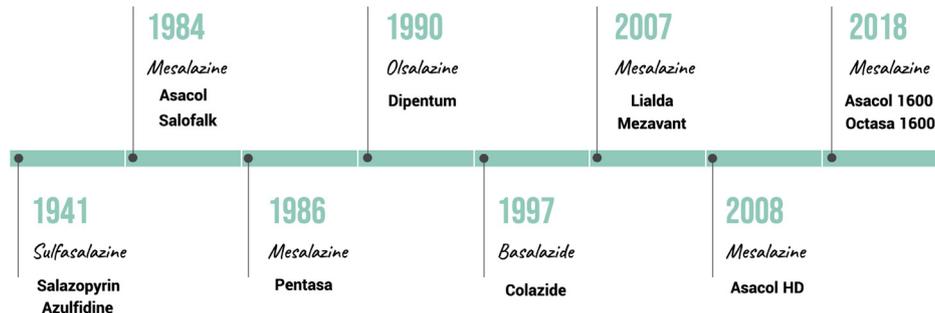
Fig. 2. Timeline showing the development of drug products for the treatment of inflammatory bowel diseases. The name of the drug are indicated in bold and brand name(s) of the product are listed below.
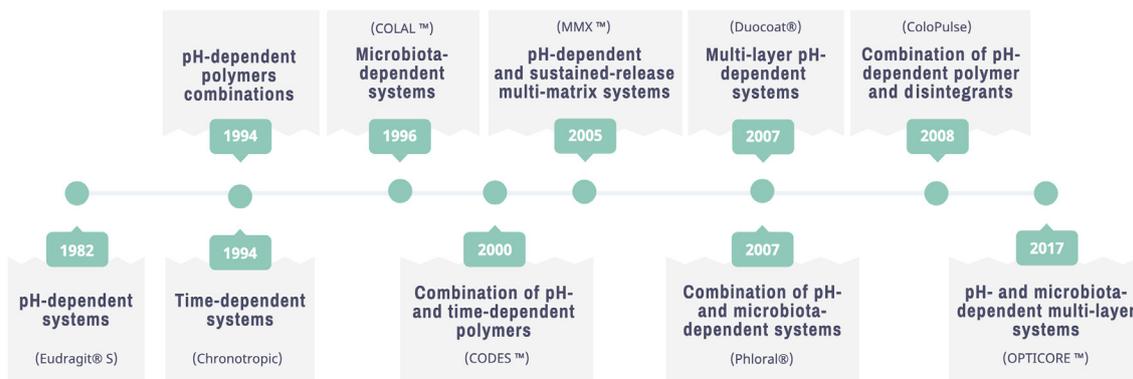
Fig. 3. Timeline of the evolution in the design of colonic drug delivery systems with key technological examples.
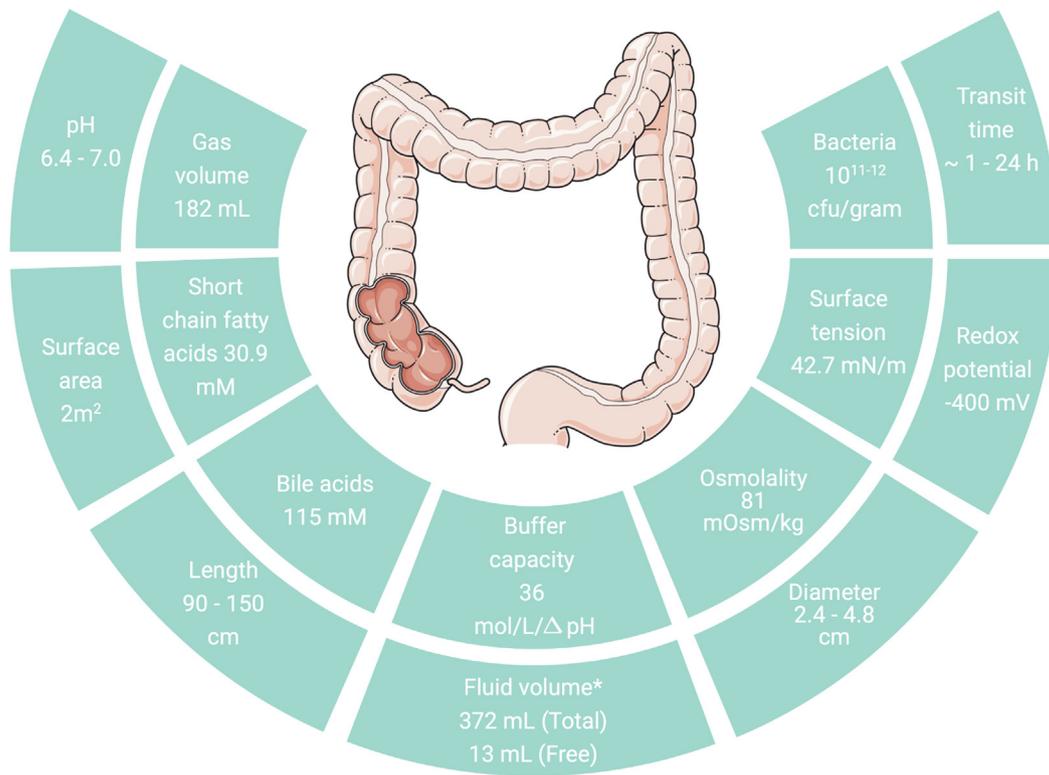
Fig. 4. Key physiological characteristics of the colon [33]. *Fluid volumes subject to free water vs. total water volumes between the fasted and fed states.

complete and accurate drug release in the colon. A timeline of the historic evolution in the design of colonic drug delivery systems with key technological examples is shown in Fig. 3.

This review provides a contemporary insight into both traditional and advanced colonic drug delivery technologies, with a clear focus on clinical and commercial translation. The unique physiology of the colon is unravelled herein, shedding light on new therapeutic opportunities, including the advanced delivery of biologics, vaccines, nutrients, chronotherapeutics, probiotics and prebiotics, and novel IBD therapies.

1.1. The colonic environment

Harnessing the therapeutic opportunities that the colonic environment holds is primarily dependent on understanding how it is formed. Basic anatomy, fluid volume and composition, GI motility and transit time, the microbiome, and intricate transporter systems are all important considerations when assessing the colonic landscape (Fig. 4) [9,30]. To achieve colon-specific drug delivery, oral dosage forms must meet certain requirements. The necessity to prevent drug release during transit through the stomach and small intestine, leading to reliable and predictable colonic delivery, is clearly overarching.

The colon constitutes only $\sim$6% (2 m^2) of the mean mucosal surface area of the GI tract, with a total length of 90–150 cm [31,32]. There are no villi in the colon and its epithelium is coated with a double layer of mucus composed of water, electrolytes, lipids and glycoproteins; the thickness of these layers varies between colonic regions, however is approximately 400–600 μm [29].

Intra-luminal fluid, or lack thereof, is a key feature of colonic physiology, especially when considering the disintegration of dosage forms, dissolution and absorption of drugs. The composition of GI fluids is dynamically changing and fluctuating, in particular with respect to the concentrations of bile salts, acid, bicarbonate, chyme, electrolytes, enzymes, gases and microorganisms [30]. Because of the high variability *between* and *within* individuals, it can be misleading to state an average colonic fluid volume. Whilst colonic free fluid volumes were estimated as 13 mL [34], the total colonic fluid volume amongst fasting healthy individuals has been projected to be 372 mL, with slightly decreasing volumes in the ascending, transverse and descending colon (as water is absorbed from faeces), although results did show considerable deviation around the means [35]. Interestingly, most water in the colon is associated with bacteria or biomass and therefore is not freely available to interact with dosage forms [36]. Free water is not homogenously distributed in the colon, but is rather located in discrete fluid pockets [37]. These fluid pockets are shown to be very small, mostly <0.5 mL, and are often primarily grouped in a single region of the colon [36]. Disintegration and dissolution of colon-targeted medicines may be highly dependent on contact with such fluid pockets. The low free water content in the colon could partly explain why the dissolution of drugs in the small intestine is often faster, and suggests that medicines targeted to the colon should be uniquely formulated with this environment in mind [38]. Once dissolved, drugs reach the systemic circulation by permeation of the double colonic mucus layer and diffusion across the epithelium (transcellularly or paracellularly), or via active membrane transporters [39]. The epithelial route taken by drugs is dependent on their physiochemistry; most drugs diffuse transcellularly and are lipophilic, whereas a minority permeates paracellularly due to their hydrophilicity. Uptake transporters, such as the peptide transporter proteins, generally favour the absorption of hydrophilic molecules [29].

GI disease can affect colonic physiology and function, and in turn affect drug behaviour and performance in patients [40-44]. For example, colonic pH is reduced in IBD patients; the epithelium

and its transporters show differences in colorectal cancer; and numerous diseases alter colonic transit time [9,45,46]. In patients with UC, the ascending colonic luminal pH is more acidic compared to healthy patients [47]. In addition, patients with IBD display up to 20% longer small intestinal transit times [48], differing fluid volumes with constipation and/or diarrhoea, a higher colonic epithelial permeability [49], colonic inflammation [50] and reduced surface mucus [51]. Furthermore, an increasing body of research has reported that systemic diseases indirectly related to the GI tract, such as cystic fibrosis, Parkinson's disease, diabetes mellitus, HIV and chronic pain, can also influence gut function [40-44]. Colon-targeted dosage forms should subsequently be designed as delivery systems which consider physiological and functional variabilities to prevent sub-optimal delivery.

2. Colonic drug delivery strategies

2.1. pH-dependent delivery systems

The pH-dependent approach to drug delivery utilises the innate pH changes along the gut. The pH of the GI tract shows variations between and within individuals and is highly affected by food intake. Whilst the lowest pH range lies in the stomach region (pH 0.4 – 4.0 in the fasted state and pH 2.0 – 4.5 in the fed state) [52,53], pH begins to rise in the proximal sections of the duodenum (pH 5.0 – 7.0) [54]. Moving from the jejunum (pH 6.6 ± 0.5) down through to the ileum, GI pH peaks at 7.5 ± 0.5 [55]. In the colon, pH drops to 6.4 ± 0.6 in the cecum and begins to gradually increase down the GI tract, reaching 7.0 ± 0.7 in the rectum. Dietary carbohydrate substrates that are indigestible to human enzymes are converted by colonic bacteria into short chain fatty acids (SCFAs) which lower pH in the proximal colon [56]. The pH increase from the proximal to the distal colon is most likely due to reduced production of bacterial SCFAs. Variations in GI pH between and within subjects do not just affect humans, but are also apparent across other species. As such, variability does not only affect the way a formulation behaves when taken by a patient, but also poses limitations when assessing efficacy in animal models [57]. Thus, not all animal species are representative of human physiology, making some unsuited for simulating human *in vivo* conditions in preclinical studies [58]. In particular, pre-clinical rat models may not be suitable to simulate the human colonic lumen for bacterial metabolism and drug stability measurements [57].

Taking this into account, it is possible to exploit the pH variations along the GI tract for targeted drug delivery. By coating formulations with an enteric polymer that disintegrates or dissolves in response to changes in the GI pH, drug release can be targeted to specific regions of the gut. Film coatings used for this type of targeting should start dissolving at a pH of around 6 – 7, and be sufficiently thick to ensure that drug release is delayed until the colon is reached (an enteric film coating needs time to dissolve after its threshold pH value is only slightly exceeded). The polymers used should be insoluble under the acidic conditions of the stomach and the proximal regions of the small intestine. In reality, most pH-mediated mechanisms release their drug cargo from the terminal ileum to the colon; as such, their delivery can be defined as ileo-colonic [59]. Examples of such pH-sensitive polymers include anionic co-polymers of methacrylic acid and methyl methacrylate [60] (e.g., Eudragit® L, pH threshold of 6; and Eudragit® S, pH threshold of 7 from Evonik, Germany). In general, these polymers have free carboxylic acid groups which remain unionised in acidic conditions, but become deprotonated once exposed to a neutral environment, rendering the macromolecules more hydrophilic and triggering their dissolution [61,62]. Dissolution rates and pH thresholds vary from one polymer to another, e.g. depending upon
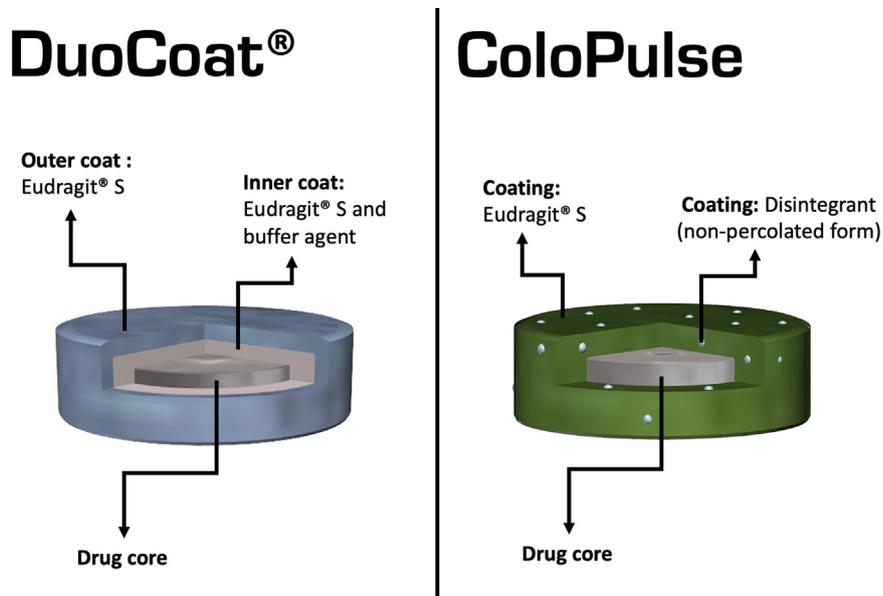
Fig. 5. Graphical illustrations showing the colon targeting strategies of the DuoCoat® and ColoPulse systems.
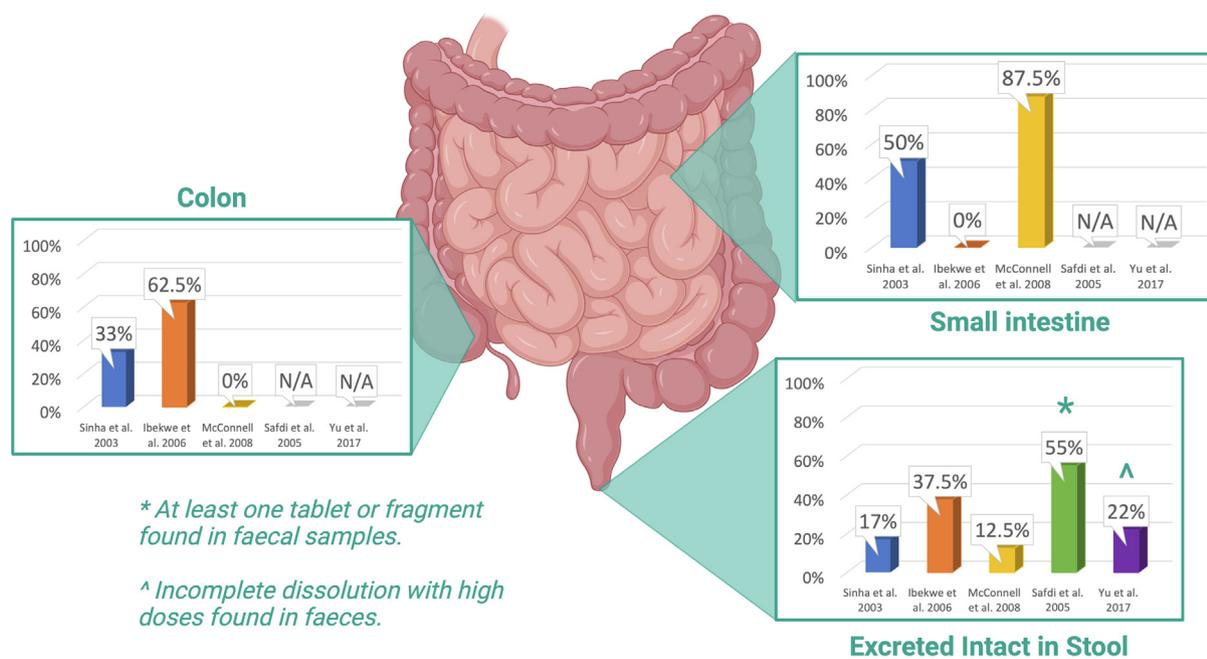
Fig. 6. Graphical analysis of the percentage of pH coatings that dissolve in different parts of the GI tract based on five human studies; Blue represents data from [82], orange represents data from [83], yellow represents data from [81], green represents data from [80] and purple represents data from [84]. Grey refers to unavailable data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the number of carboxylic acid moieties per molecule [63]. Typically, the higher the number of carboxylic acid groups in a polymer, the lower its pH threshold and the faster its dissolution rate at neutral pH (and vice versa) [64,65].

The first attempt to utilise pH-dependent polymers for ileo-colonic delivery was in 1982 [66]. The enteric polymer Eudragit® S was employed as a capsule coating and studied in patients using X-ray imaging. The majority of capsules were seen to disintegrate in the terminal ileum and proximal colon [67]. These studies paved the way for the development and commercialisation of several Eudragit® S-coated formulations, including Asacol® MR, Mesren® MR and Ipocol® [68].

Following the widespread use of single layer pH-dependent polymer coatings, double-layer systems were launched onto the market. DuoCoat® (Evonik, Germany) exemplifies such drug delivery technology (Fig. 5). DuoCoat® incorporates two pH-sensitive coating layers; the inner is composed of partially neutralised Eudragit® S combined with a buffer agent [e.g., citric acid/KH_2PO_4/$(NH_4)_2CO_3$], and the outer of solely Eudragit® S [69,70]. Compared with single-layer pH-dependent coatings, double-layer systems often achieve a shorter dissolution lag time and higher drug release rates once the solubility pH threshold is reached [71,72]. In the case of DuoCoat®, the polymer coating can dissolve from both its inner and outer surfaces. As intestinal fluid gradually penetrates
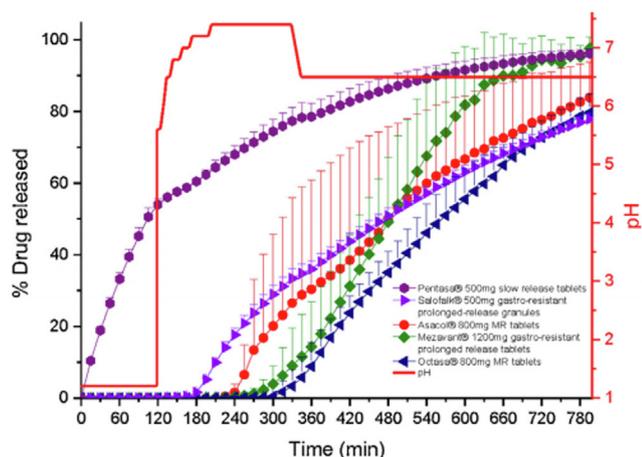
Fig. 7. Drug release profiles from commercial mesalazine formulations in 0.1 M HCl for 2 h followed by physiological bicarbonate buffer under dynamic pH conditions (pH ramp from 5.6 to 7.4 followed by a drop to pH 6.5) controlled by the Auto pH System™. Red line shows real-time pH dissolution values. Reprinted with permission from [90]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

through the outer coating, the inner coating rapidly dissolves and produces a dynamic internal environment of high buffer capacity, expediting the dissolution of the outer polymer [69].

Another attempt to improve upon pH-mediated delivery includes the ColoPulse system, which as the name suggests, aims to achieve ileo-colonic delivery via pulsatile drug release [73,74]. The system comprises a disintegrant (e.g. sodium starch glycolate, croscarmellose sodium, microcrystalline cellulose or alginic acid) incorporated in a pH-sensitive polymer (e.g. Eudragit® S) in a non-percolating form (where no continuous network of disintegrant particles is created). Once the pH threshold of the polymer is reached, considerable amounts of fluids penetrate the coating. The presence of the disintegrants accelerates the disruption of the film coating, and a more pulsatile drug release profile is achieved. A potential limitation of the ColoPulse system, however, is that an organic-based coating is required to avoid premature swelling of the superdisintegrant [75,76].

A recent example of ileo-colonic targeting for IBD treatment, utilising pH-sensitive polymers, is IBD98-M, a formulation designed to treat mild to moderate UC by local delivery of 200 mg 5-ASA and 23 mg sodium hyaluronate. It consists of a capsule filled with multiple enteric-coated pellets designed to release the active ingredients at pH 6.8 or above once the terminal ileum is reached. The pellet core, based on microcrystalline cellulose, is coated with a primary layer of 5-ASA, a secondary layer of sodium hyaluronate, and an outer film of enteric polymer. Clinical studies evaluating the safety and efficacy of IBD98-M in 51 patients found that although IBD98-M did not meet the primary end-point, patients had higher clinical responses, with significant reduction in inflammation biomarkers (e.g. faecal calprotectin) and a significant improvement in quality of life, compared to a placebo [77].

Despite the clinical and commercial success of traditional pH-dependent polymers, their colon-targeting efficacy has demonstrated distinct patient variability. The inaccuracy of Eudragit® S-coated tablets to disintegrate in the colonic environment has been reported by several sources (Fig. 6) [59,78–80]. Common reasons of coating failure include premature drug release occurring in the small intestine and insufficient coating dissolution/degradation in the colon (identifiable by intact tablets in patients' stools). These shortcomings have been attributed to a myriad of physiological factors, including feeding status, fluid volumes, gut motility, buffer capacity and ionic strength of GI fluids [81]. These are all features

known to differ between and within individuals. Clearly, formulation failure to deliver drug to the intended site of release is critical; potentially forming the difference between treatment success and failure.

The market currently offers several pH-sensitive formulations loaded with mesalazine such as Lialda®, Asacol®, Octasa®, Pentasa® and Salofalk®. Despite containing the same active, evidence has shown that some patients may actually benefit from switching to a different type of formulation after an inadequate response to a previous treatment [85]. This is probably because each formulation has a different drug release profile. During the preclinical evaluation of a release performance, it is essential to develop an accurate *in vitro* model that best represents the physiological conditions in the human GI tract. *In vitro* dissolution testing media often includes phosphate buffer as representative of small intestinal fluid composition. However in reality, bicarbonate is the main buffer species in the human GI fluid, not phosphate [86–89].

For this reason, bicarbonate buffer is more physiologically relevant and represents a promising alternative to the compendial phosphate buffer, being able to better discriminate the behaviours of oral dosage forms [71,89]. This concept is best exemplified by a study which employed bicarbonate buffer [90] in a dynamic *in vitro* model that simulates the intestinal conditions using an Auto pH System™ [75,91,92]. The drug release performance of different mesalazine products was compared, wherein each drug product exhibited a distinct dissolution pattern (Fig. 7). Notably, the release profile of the Lialda® (Mezavant® XL) product displayed a close correlation with gamma-scintigraphy data in humans [93], demonstrating the reliability of the bicarbonate buffer in mimicking human conditions *in vitro*.

2.2. Time-dependent delivery systems

Time-dependent systems attempt to utilise the time delay between dosage form ingestion and colonic arrival to achieve colon-specific targeting. However, whole gut transit is highly variable, whereby movement through regions occurs at intervals [94–97]. In the colon, rhythmic contractions mix intraluminal contents and can propagate into high-amplitude propulsions that move contents in defined bursts, enabling defecation [29]. However, several factors play a role in gut motility and transit. An example of such is biological sex. Females are reported to have significantly delayed gastric emptying, and longer whole gut and colonic transit times compared to males [95,98–100]. In terms of differences in the residence times within *regional* colon sections, females may have longer transits through the *transverse* colon and *descending* colon, but shorter transit in the *rectosigmoid* part [94]. These sex differences have largely been attributed to the interplay and action of endogenous hormones, though further research is needed to validate and quantify these effects [100,101]. Age and body mass index (BMI) also have significant effects on GI motility and transit. Increasing age is associated with longer whole gut, colonic, ascending colon, transverse colon and total right colon transit times, but shorter rectosigmoid times [94,97]. Age-associated risk factors for slow colonic transit include polypharmacy (i.e., the concomitant use of five or more drug products), decreased fibre intake, and lower levels of physical activity [102]. Increasing BMI has been shown to decrease whole gut transit and gastric emptying time, and potentially increase colonic transit time [94,103,104]. Rapid gastric emptying has the propensity to reduce negative feedback satiety signals, thus increasing the risk of overeating [104]. Less is known about the relationship between colonic motility and obesity [105].

The time-based formulation approach for targeted drug delivery to the colon relies on average GI transit times. Despite the high fluctuation and inconsistency in the gastric residence time, time-
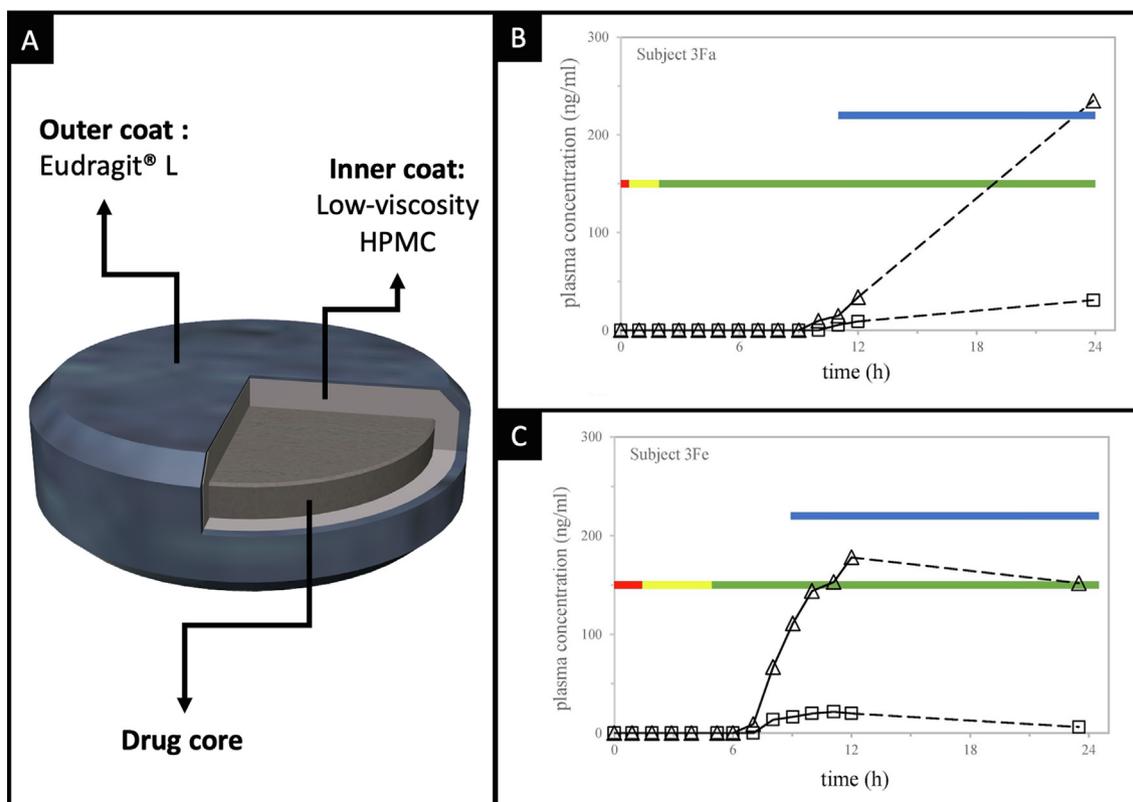
Fig. 8. (A) Graphical illustration showing a 5-ASA dual time-dependent coating system. Plasma concentrations of (□) 5-ASA and (△) N-acetyl5-ASA following administration of the dual time-dependent coating system to a patient in the (B) fasted and (C) fed state. Red, yellow and green bars represent gastric, small intestinal and colonic residence respectively; the blue bar represents disintegration. Partially reprinted with permission from [114]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dependent systems should remain completely intact when in the stomach region. Once in the duodenum, the rise in pH may initiate a lag phase but no major drug release should occur. This triggering signal can be achieved through the application of an outer enteric coating, whose dissolution should be equivalent to or surpass that of the average small intestinal transit time [106]. Theoretically, such an effect can be achieved using any approach that results in a predefined delay interval. Regardless of the strategy used to delay the drug release, these systems should have a consistent lag phase and reproducible effect. To ensure that, sealing plugs or layers composed of swellable and erodible polymers are often used to isolate the drug-laden regions or to cover the interior of the drug reservoirs [107,108]. In fact, the majority of time-based dosage forms intended for colonic drug delivery were initially developed for chronotherapy in the form of pulsatile drug release [106].

Systems for time-controlled colonic targeting can conveniently be grouped as reservoir, capsular and osmotic devices. Reservoir systems can in turn be differentiated based on the functional characteristics of their coating layer, which may function as a rupturable, erodible or diffusive barrier. Reservoir systems with a diffusive membrane represent one of the first attempts to design a time-dependent drug delivery system. Several examples are reported in the literature, mainly dating back to the late 1990s [109-112]. In these systems, the formulation trigger phase coincides with full water penetration through the diffusive external layer, occurring after upper GI transit. For instance, one study proposed a system in which the drug was incorporated into a core formulation and subsequently coated with a mixture of Eudragit® RS and channelling agents (e.g. NaCl and Emdex® binders [112,113]. Drug release from these dosage forms should be delayed until the channelling agents are dissolved, creating diffusion

pathways for the drug. The type and particle size of the investigated channelling agents as well as the composition of the core are reported to influence the lag time and release rate of the drug. Gazzaniga and colleagues suggested a coating of immediate-release 5-ASA tablets with two layers: An inner coating based on a low-viscosity hydroxypropyl methyl cellulose (HPMC), and an outer coating based on Eudragit® L30D (Fig. 8A) [114]. *In vitro* data and a pharmacoscintigraphic study involving 6 patients have shown that whilst the enteric film coating safeguarded against drug release in the stomach, the HMPC coating ensured a reproducible lag phase once neutral pH values were encountered, providing drug release in the colon (Fig. 8B and C).

The main limitation of time-dependent colonic delivery strategies is their reliance on predictable GI transit. As mentioned previously, GI transit is highly variable between and within individuals making accurate prediction very difficult, especially in disease states. This also applies to reliance on pH gradients as triggers to begin lag phases; where such gradients do not exist, there is more unpredictability.

2.3. Colonic microbiota-dependent systems

Since the culmination of the human microbiome project in 2012, it has been known that commensal bacteria encode for 150-fold more unique genes than their human host, and greatly contribute towards health and disease [5,115,116]. The majority of the 100 trillion microbial cells in the human microbiome reside in the gut, with the highest concentration of 10^{12} bacteria per gram in the colon [117,118]. These bacteria wield significant metabolic power that can be harnessed for drug delivery. For example, prodrugs can be activated by colonic bacteria. Sulfasalazine represents

the earliest example of a prodrug that relies on colonic bacteria for activation to its active moiety, 5-ASA [119]. Colonic microbiota can also facilitate drug release from formulations, for example by coating dosage forms with microorganism-sensitive materials. Such materials include polysaccharides, which can be degraded by colonic bacteria through enzymatic fermentation to lactate and SCFAs [120]. Though individual microbiome compositions are unique, general functions such as polysaccharide digestion are performed similarly in most of the population due to substantial functional redundancy amongst microbiota, thus polysaccharides are largely reliable materials for colonic drug delivery [121]. Whilst the metabolic power of the microbiome can be exploited for drug delivery, it can also hinder the delivery of certain therapeutics due to their extensive microbial metabolism resulting in decreased bioavailability [122]. Furthermore, toxic degradants may be generated by microbial drug metabolism, as is the case for the antiviral brivudine which is transformed to a hepatotoxic metabolite, bromovinyluracil, by the *Bacteroides* species [123].

Microbiota-dependent systems have been widely explored for targeted drug delivery to the colon [26]. Certain polymers are indigestible in the proximal gut but are selectively metabolised by colonic bacteria, therefore representing attractive coatings for colonic release dosage forms [124-128]. To date, two main classes of polymers have been explored for use: azo-polymers and polysaccharides [128-132]. Due to the carcinogenic potential of azo-polymers, and the need to use organic solvents in their preparation, further use in humans has been halted [132]. As such, polysaccharide-based formulations are the key facilitators of enzyme-sensitive systems for colonic drug release. Examples of naturally occurring polysaccharides employed for colon targeting include pectin, starch, alginate, gums, amylose, chitosan, dextran, chondroitin sulphate, inulin, β-cyclodextrin and galactomannan [133-138]. The most frequently employed ones will be henceforth discussed in further detail.

2.3.1. Pectin

Pectin is an α-D-galacturonic acid-rich heteropolysaccharide present in plant cell walls [138]-. A coat of a high methoxygrade pectin around a core of sodium fluorescein has been investigated as a model colon-targeted formulation [139]. The tablets were studied *in vitro* in simulated mouth to colon conditions and *in vivo* in human subjects. The system showed favourable results where *in vivo,* all tablets disintegrated in the colon after ~5.5–8.8 h, with exact disintegration regions varying from the caecum to the splenic flexure. However, the need for large quantities of pectin (700 mg) to coat a small tablet core (120 mg) has rendered pure pectin coatings inefficient for scale-up and inacceptable to patients due to large tablet size. As an alternative, other approaches utilising pectin's calcium salt or mixing pectin with other components have been investigated [140-149].

A scintigraphic evaluation of pectin–HPMC tablets, conducted with six healthy male human volunteers, demonstrated that the combination successfully passed through the proximal gut intact and degraded in the colon [149]. Pectin has also been used as a matrix in fluorouracil microspheres coated with Eudragit® S100 [150] or in a blend with ethyl cellulose (EC) [146]. A noteworthy pectin/EC blend has been used in the SmPill® system, a colon-targeted cyclosporine A formulation developed by Sublimity Therapeutics (formerly known as Sigmoid Pharma) [151]. In pigs, the SmPill® system demonstrated enhanced delivery and uptake in the colon for the treatment of local inflammatory conditions. Despite reaching Phase II clinical trials, the system did not show favourable results, which led to the abandonment of this approach. In another example, pectin was combined with cellulose acetate and a layer of Eudragit® L100 to form a microporous bilayer osmotic tablet [147]. Pectin also plays a key role in Samyang Biopharm's formulation for treatment of UC. In this marketed product, prednisolone is embedded in a tablet core and coated with a primary layer of enteric polymer and an external layer of pectin hydrogel [152].

2.3.2. Guar gum

The most commonly used gum for colonic drug delivery is guar gum. The latter is a naturally-occurring galactomannan polysaccharide found in the endosperm of *Cyamopsis tetragonolobus* [153,154]. The high viscosity of guar gum renders it stable in the gastric and small intestine regions. This polysaccharide has been abundantly studied for its potential use for colon-specific drug delivery, both as a matrix polymer [155-160] and as a coating [160-163]. A pharmacoscintigraphic approach was used to examine the *in vivo* performance of three dexamethasone formulations compromised of guar gum matrices [157]. Each formulation displayed varying disintegration and drug release characteristics. Interestingly, while between 72–81% of the drug was released in the colon, the remainder of the drug (approximately 20 –30%) was liberated in the small intestine. This highlights a major drawback of matrix formulations as opposed to coated solid dosage forms, especially in the case of readily soluble drug agents.

2.3.3. Chitosan

Chitosan is a high molecular weight, polycationic polysaccharide derived from naturally-occurring chitin, extracted from the exoskeleton of insects and shellfish [163]. Azathioprine-loaded chitosan beads have been studied in acid-induced colitis rabbit models as potential treatments for IBD [164]. The beads demonstrated good colonic targeting, whilst improving therapeutic outcomes compared to non-targeted controls. An interesting characteristic of chitosan is its mucoadhesive properties arising from interaction with mucin through hydrogen and electrostatic bonding [163]. Mucoadhesive colonic formulations could be advantageous for treatment of CD, in which GI mucus production is increased [165,166]. *In vivo* studies in rats have demonstrated significant accumulation of 5-ASA-loaded chitosan pellets in diseased colonic tissue due to muco-adhesion [167].

A hybrid coating of chitosan, pectin and HPMC has been explored for colon-specific drug delivery [128,168]. Scintigraphy has shown that tablets coated with such a blend remain intact as they move through the stomach and small intestine of human volunteers, and readily degrade in the colon [128,168]. Chitosan has also been combined with locust bean gum for colonic delivery, offering further colon-targeted coating opportunities [169].

2.3.4. Starch

One of the most recent and promising types of excipients for targeted colonic delivery are resistant starch and its derivatives [170,171]. Typically, starch polymers are built from two main components, amylopectin and amylose, with the latter constituting 15–25% of the total polymer weight [172]. Whilst starch degradation mainly occurs in the small intestine by pancreatic enzymes, some of its forms remain unaffected and are known as *resistant* starch [171,174-180]. Resistant starch can be classified into four types: physically inaccessible, ungelatinised, retrograded and chemically-modified. Despite the differences between them, all types of resistant starch remain intact in the small intestine, making them an accessible source of energy for colonic bacteria [176].

Of the different types of retrograded starch, the glassy amorphous amylose has shown to be unaffected by pancreatic enzymes, defining its value as a colon-targeted coating [178,180]. This type of amylose has shown to be fermentable by more than half of the microbiota population, and therefore when purposed as a drug delivery system, it demonstrates uniform release across
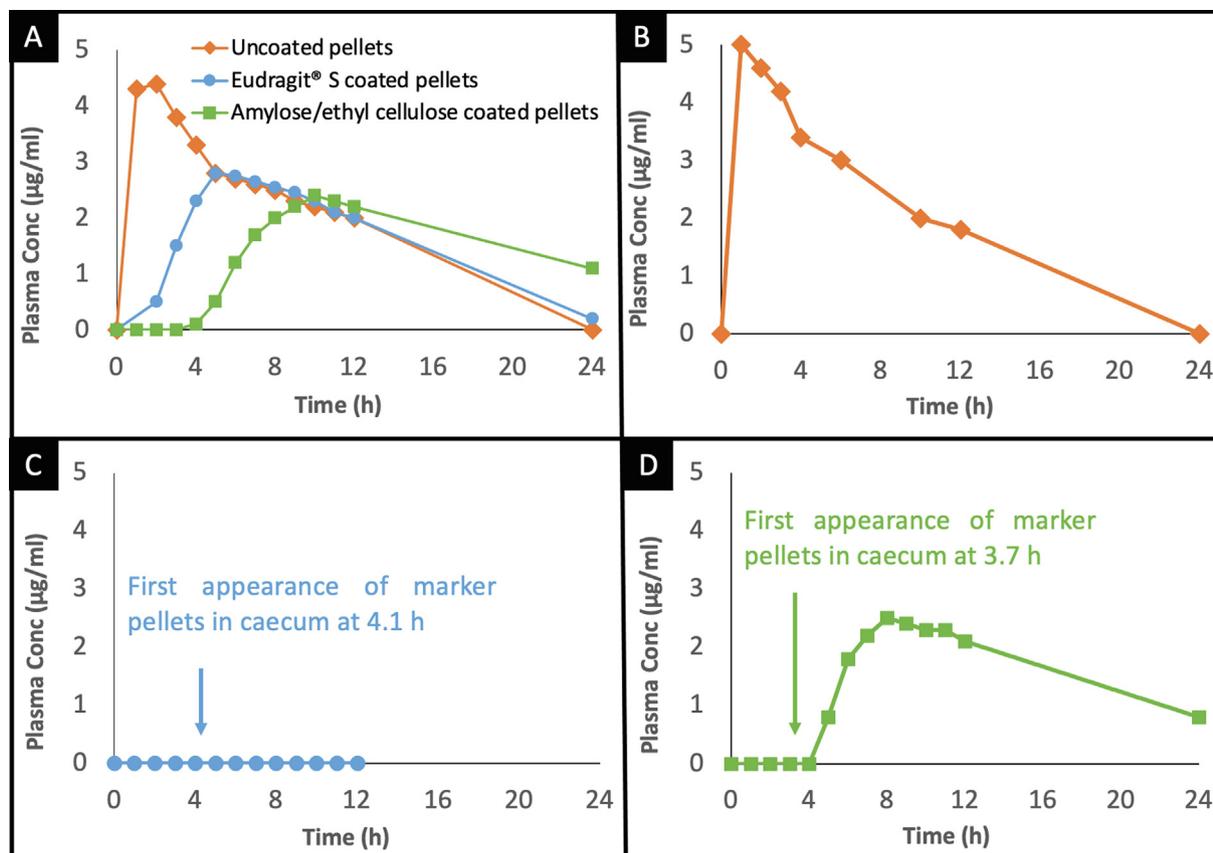
Fig. 9. (A) Mean plasma theophylline levels after administration of uncoated, Eudragit® S coated pellets and amylose/EC coated pellets to eight healthy male subjects. B, C and D show examples of plasma concentration time profiles observed in single subjects: (B) uncoated pellets; (C) Eudragit® S coated pellets and; (D) amylose/EC coated pellets. Reprinted with permission from [81].

individuals as coating digestion is not reliant upon a select strain of microbiota [175].

Due to the swelling effect of amylose in the presence of water, it is often combined with a water-insoluble polymer, such as EC, to avoid premature drug release in the upper GI tract [181,182]. An *in vivo* study in humans directly compared a starch-based coating (amylose + EC blend) with a pH-dependent coating (Eudragit® S), for colonic drug delivery [81]. The study aimed to examine the ability of both materials to target drug release to the colon. Theophylline-loaded pellets were coated with either material, with uncoated pellets acting as an untargeted control. The starch-based coating showed more specific colonic release compared to the pH-sensitive formulation. The amylose/EC coated pellets were seen to only release drug when located within the colon, whereas pH-triggered pellets were suspected of premature drug release in the small intestine. The Eudragit® S coated pellets also demonstrated broad inter-patient variability, with a complete absence of drug release in one patient (Fig. 9).

This microbiota-sensitive coating formed the basis for a new treatment for UC: COLAL-PRED®, a colon-targeted tablet loaded with prednisolone metasulfobenzoate sodium [183]. As prednisolone metasulfobenzoate sodium has very limited systemic absorption, its therapeutic activity may be targeted to the colon when released locally. In clinical trials, the COLAL-PRED® system demonstrated effective treatment of UC by achieving dose-dependent improvements in disease activity and severity in mild to moderate cases. Improvement of patients' symptoms and overall clinical outcome occured after 4 weeks of high-dose treatment, followed by 3 weeks of maintenance dosing. Phase III clinical trials in UC patients showed that whilst the formulation did not display

higher clinical efficacy over traditional oral dosage forms of prednisolone, it achieved superior safety records.

The activity of microbiota-triggered systems may be impacted by several factors. As an example, these systems may exhibit reduced effectiveness in patients receiving antibiotic therapy [184]. This is because the use of antibiotics decreases the abundance of colonic microbiota, lowering their enzymatic activity that is primarily responsible for the activation of enzyme-sensitive systems. Another limitation of these systems is associated with the hydrophilicity of polysaccharides, causing them to significantly swell in the proximal gut when used in isolation. Therefore, polysaccharides such as starch are often combined with an insoluble matrix, most notably EC, which acts as a structuring agent that helps reduce the swelling effect [182,186]. However, this requires the use of a thick EC coating that consequently slows down the drug release. Furthermore, as amylose is the digestible component in starch but accounts for no more than 15–25% of its total weight [172], the ratio of amylose to EC plays a major role in controlling the drug release rate.

3. 3D printing: A novel pharmaceutical approach

Three-dimensional (3D) printing offers an alternative way to create dosage forms with customised properties for colonic drug delivery. Transitioning away from traditional production methods, pharmaceutical research has recently explored the use of novel fabrication systems which could decrease inter- and intra-individual variability in medication response due to the scope for personalisation. An example of such is 3D printing, which encompasses several additive manufacturing processes with the ability to

create 3D objects from a computer-aided design (CAD) model in a layered fashion [186,187]. Due to the digital nature of the process, it is easy to create customised dosage forms and drug-eluting devices [188-192] with different shapes, sizes and drug content [192-195]. This permits healthcare professionals to design and dispense personalised drug products that meet the individual needs of each patient [192,195,196]. This ensures that each patient receives an efficacious drug dose, reducing chances of ineffective treatment or incidence of adverse events and avoiding unnecessary hospitalisations [197].

In the last decade, 3D printing has been increasingly investigated to produce a wide array of novel 3D printed drug-laden formulations, termed as Printlets™, which can have complex designs that would be challenging or infeasible to create using traditional production technologies. Examples of such include Printlets with tailored release patterns [198-201] and 3D designs [201-205] that span controlled release, fast dissolving [205-209] and multi-drug dosage forms [209-213]. Accurate control of drug release can be achieved by fine-tuning different parameters [193,213,214], such as geometry [215,216], infill percentages [i.e., degree that the internal structure of a Printlet is filled with material which, ranges between 0% (creating a hollow Printlet) to 100% (an entirely solid Printlet)] [217,218] or composition [219,220]. Several studies have evaluated this concept for single drugs as well as polypills [209,221] (products containing multiple active ingredients). With 3D printing, both the shell and infill can be created using a single process [222,223], or alternatively drugs can be incorporated directly within an enteric matrix, thus negating the need for an external enteric shell [224]. As digitisation is an intrinsic feature of 3D printing, it is easily combined with other advanced digital health technologies, such as artificial intelligence, machine learning (ML) [225-228] and smartphone technologies [228], which further supports the adoption of personalised medicines as part of a novel healthcare model [229,230]. Such a model would involve the integration of a closed-loop system that could combine health monitoring, disease diagnosis, drug manufacturing and dispensing [231].

Numerous attempts have been made to exploit 3D printing for the treatment of colonic diseases. Examples include the fabrication of modified-release budesonide Printlets for the treatment of IBD [232]. Herein, the Printlets were coated with an enteric polymer following the printing process, using a fluid bed coater to achieve delayed drug release. Similarly, the suitability of 3D printing for the fabrication of both the shell and drug-loaded core of a Chronotopic™ colonic system has been demonstrated [233]. Several configurations of the system were designed and studied. In a different approach, 3D printing was explored for the on-demand preparation of tacrolimus suppositories based on the dose requirements of patients from different age groups [234]. Tacrolimus is an immunosuppressant drug with a narrow therapeutic window, thus the ability to personalise dosing, dependent on individual drug plasma concentrations, provides a more reliable approach for the safe dispensing of this medication [235]. The 3D printed suppositories were tested in an IBD rat model and demonstrated quick disintegration times and favourable disease remission results [236]. Another distinctive study involved an advanced fabrication method that combined 3D printing with injection volume filling for drug delivery to the colonic region. The dosage forms were fabricated using a single system that allowed the 3D printing of scaffold structures and subsequently their filling with a liquid or semi-solid matrix [237].

4. Multi-faceted approaches to colonic targeting

In isolation, all colonic release strategies have their limitations and taking into account the variability in the gut, single-triggered systems may not always achieve a consistent therapeutic effect. By including a secondary or backup trigger in targeted formulations, there is an opportunity to improve their reliability by increasing their chances of successfully releasing drug in the colon. Possible combinations of colonic drug delivery mechanisms are manifold, incorporating all types of pH-, time-, and microbiota-dependent systems. Depending on how they are designed and when their triggering mechanism unfolds, multi-faceted approaches have been sub-classified into either sequential or parallel triggers in the context of this review. Some of the most successful of these combination systems will be discussed herein.

4.1. Sequential triggers

Herein, "sequential triggers" is an umbrella term used to refer to multi-matrix approaches where the triggering action is exerted in a consecutive order. Such systems are typically in the form of multi-layers, wherein the outermost layer should first solubilise before the following layer can exert its action.

4.1.1. pH- and time-dependent combinations

The Multi Matrix System® (or MMX®) is a system that combines a pH trigger mechanism along with a time-controlled release strategy [238]. The drug, i.e. mesalazine [Lialda® (Mezavant® XL)] or budesonide [Cortiment® (UCERIS®)], is incorporated in small lipophilic matrices which are embedded within a larger hydrophilic matrix. This "dual" matrix system is surrounded by an enteric film coating (e.g. based on Eudragit® L and S). In theory, upon dissolution of the enteric coating at neutral pH, the "dual matrix" is expected to slow down drug release. Interestingly, although the release of 5-ASA was shown to be sustained for more than 8 h (upon the onset of initial drug release) and followed zero- order kinetics in phosphate buffer, the release patterns were substantially different in Krebs bicarbonate buffer [86,90]. In fact, 5-ASA release in bicarbonate buffer was more instantaneous and much more similar to that of "conventional" pH-sensitive colon targeting systems (e.g., Asacol® and Ostasa®). Also in human subjects the MMX® technology exhibited similar 5-ASA pharmacokinetics as a "pH-dependent, delayed-release mesalazine" [239]. A similar example is represented by the Budenofalk® product which is composed of granules with a dual coat; the inner layer is a mixture of Eudragit® RS, Eudragit® L100 and Eudragit® S and the outer coat is comprised of Eudragit® RL [75].

Another example is the Ethyl Cellulose Matrix (or ECX™) which has been used within the multi-particulate dosage product Entocort™ EC. The system is designed to deliver the corticosteroid budesonide to the ileo-colonic region to treat CD and UC. Here, the system consists of a hard gelatine capsule containing 3 mg of the drug in pellet form. The pellets have an inert saccharose core coated with an inner layer of insoluble EC and an outer layer of Eudragit® L100-55 [76]. Human pharmacoscintigraphic studies reported that a significantly greater fraction of budesonide was delivered to the ileo-colonic region, compared with the standard capsules [240]. In addition, phase III clinical trials showed that clinical remission was significantly higher for patients taking Entocort™ EC compared with the placebo group [241].

Recently, Eudratec® COL was developed. This system is composed of an outer layer of Eudragit® FS 30D coating and an inner layer of Eudragit® RL or Eudragit® RS. The latter two substances contain quaternary ammonium groups with chloride counter ions, enabling them to behave as diffusion-controlled membranes [242]. *In vivo* evaluation of caffeine pellets coated with an inner layer of Eudragit® RL/RS 30D and an outer layer of Eudragit® FS 30D reported ileo-caecal delivery, with a prolonged serum caffeine profile, compared with a pH-only system [243].

Phloral® dual mechanism
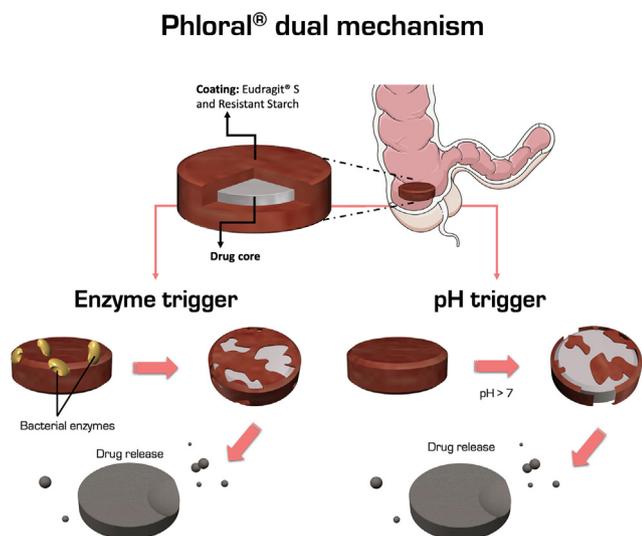
Fig. 10. Composition and mechanism of action of the Phloral® coating.

4.1.2. Time- and microbiota-dependent combinations

Formulations harnessing time and enzyme sensitive colonic delivery formulations have only recently begun to be developed. One such example exploited the combination of high amylose starch and HPMC of different chain lengths [244]. The HPMC starts to swell and degrade when in contact with GI fluid, generating the lag time to reach the colonic region. Once the capsule reaches the colon, enzymes produced by colonic bacteria digest the starch portion, leading to a fast and complete release of drug cargo. These capsules have been loaded with paracetamol, and *in vitro* methods demonstrate faster pulsatile drug release when the formulation is in the presence of faecal microbiota. This highlights how both elements of the release system play an instrumental role in drug delivery and the synergy that results from the combined approach.

4.1.3. pH- and microbiota-dependent combinations

An example of a sequential pH and bacteria-sensitive system is the multilayer structure CODES™ [245]. The tablet core consists of lactulose surrounded by Eudragit® E coated with Eudragit® L. After ingestion, the outermost Eudragit® L coating protects the tablet from the acidic gastric fluid, and subsequently dissolves upon entry to the small intestine. Once in the colon, the lactulose component of the tablet begins to be fermented by bacteria. SCFAs produced during fermentation induces the dissolution of the acid-soluble Eudragit® E coating, exposing the tablet core and prompting drug release [246,247].

Whilst sequential triggering systems combine more than one mechanism, in reality, they do not actually circumnavigate the risk of drug release failure nor provide a significant improvement in the reliability of the formulations. This is because the outermost layer will still be the rate-controlling step and if the critical threshold needed to trigger its dissolution is not reached in the patient's gut, the inner matrix may not be triggered and the system is likely to exhibit variability in its drug release pattern.

4.2. Parallel triggers

"Parallel triggers" on the other hand, are multi-faceted approaches encompassing two triggering mechanisms combined within the same matrix. As such, the triggers function independently from one another. In fact, both mechanisms can function simultaneously as long as their triggering threshold is reached. In the case where one of the triggers fails to be activated, the action of the other trigger can compensate and the drug product is still released in the colon as intended. Thus, the use of parallel triggers provides a "fail-safe" effect, where the drug delivery to the colon is more reliable.

A particularly promising colonic delivery strategy based on parallel triggers is the combination of pH and enzyme triggers. Indeed, the past couple of years have witnessed the commercialisation of two such technologies, which will be henceforth described. In both cases, the colon-targeted coatings have facilitated the local delivery of therapeutics for IBD, however should be applicable to the delivery of a large variety of drugs.

4.2.1. Phloral®

The first successfully marketed combination technology, Phloral® [248,249], intermixes Eudragit® S and resistant starch in a single coating layer, wherein both mechanisms complement one another to provide fail-safe colonic drug release (Fig. 10) [250]. Whilst the pH-responsive polymer protects the integrity of the tablets in the proximal gut and regulates starch swelling, the addition of resistant starch adds a secondary trigger for drug dissolution and release, should the critical pH value needed to dissolve the pH-dependent polymer not be reached. Demonstrating the potential of this dual mechanism approach to be used for systemic treatment or local therapy in the colon (e.g. IBD) due to its high site-specificity, Phloral® has shown favourable results in all feeding conditions (fasted, pre-fed and fed states) [250].

4.2.2. OPTICORE™

An even more recently marketed technology is OPTICORE™, a dual-triggered system applied to successfully deliver 5-ASA to the colon for treatment of IBD [251]. The name OPTICORE™ stands for OPTImised COlonic RElease, demonstrating the purpose of the formulation. To achieve optimised colonic drug delivery, OPTICORE™ combines an inner alkaline coat (including a neutral enteric polymer, such as Eudragit® S and a buffering salt) with an external Phloral® coating to enhance further colonic specificity (Fig. 11).

In the OPTICORE™ system, several characteristics of the colonic fluid modulate drug release: pH; buffer capacity; buffer salt concentration; ionic strength and viscosity. The coating ensures early and rapid drug release in the ileo-colonic region where fluid is more abundant than in the more distal areas of the colon. As the outer coat starts to dissolve or is fermented by bacterial enzymes, fluid enters the formulation through emerging pores in the coating, resulting in dissolution of the inner coat. This generates an environment with elevated pH, buffer capacity and ionic strength, at the inner surface of the Phloral® coating layer. Consequently, the Eudragit® S in the Phloral® coat undergoes rapid ionisation and dissolution, expediting drug release [70,252,253]. A recent phase I clinical trial used the OPTICORE™ technology for the targeted delivery of metronidazole benzoate to treat localised colonic *Clostridioides (formerly Clostridium) difficile* (*C. diff*) infection. Accurate ileo-colonic targeting was achieved with reduced systemic concentrations, compared to immediate-release findings [254]. Additionally, Asacol™ 1600 mg, a 5-ASA product based on the OPTICORE™ technology, has successfully passed Phase III clinical trials and been marketed in Europe [255]. Owing to its high colonic specificity and reliability, this dual-trigger system allows the incorporation of up to 1.6 g of 5-ASA; large doses of 5-ASA (up to 4.8 g daily) are required to achieve the recommended doses in mild to moderate UC cases. This ability to deliver a substantial dose in a once daily formulation reduces the frequency patients must take doses, thus improving patient compliance and acceptability.

Whilst their initial development was for the treatment of local colonic diseases, the clinical success of the dual trigger of the Phloral® and OPTICORE™ systems has rendered them as universal delivery systems that enable the transport of virtually any product to
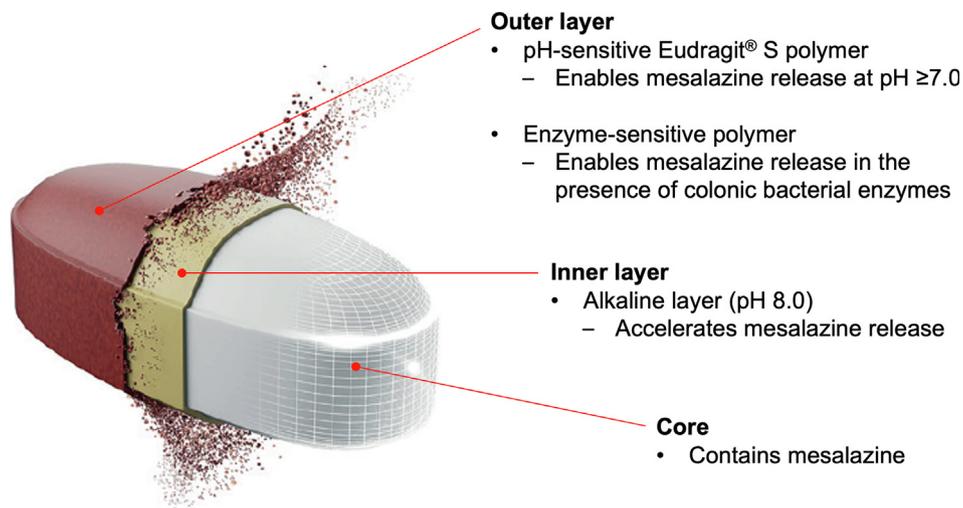
Fig. 11. Graphical illustration of the Asacol™ 1600 mg commercial drug product, explaining the OPTICORE™ coating system. Reprinted with permission from [256].

the colon. This paves the way for their use for treatment of diseases beyond IBD and local diseases of the colon.

5. Harnessing the colon for advanced drug delivery

With state-of-art technologies now available, a myriad of opportunities to exploit the colon can be unveiled. Within the last five years, research has uncovered and characterised how diseases can alter colonic physiology, and the subsequent ramifications for drug efficacy [257]. Similarly, extensive work has been conducted on understanding how the colonic environment may differ in special populations, such as paediatrics, pregnancy, and even between sexes and ethnicities [46,258]. Such information is highly useful when moving towards specific and personalised therapies that optimise drugs' pharmacokinetics. A field experiencing intensive growth is research encompassing the microbiome, wherein genomic sequencing of the colonic microbiota has allowed detailed analysis of commensals' relationship with diseases, drugs and pharmacokinetics [5,122,259]. Such knowledge can be applied to ameliorate the colonic microbiome for prevention and treatment of diseases [120,260]. Chronotherapy is an additional field casting light on the potential benefits of colonic drug delivery. Almost the entire human genome is regulated by circadian rhythms, thus, rationalising the concept of adapting drugs to work in harmony with the body's natural cycles [261]. In fact, the colon serves as an ideal site for delivery of chronotherapeutics because long retention times allow drugs to be programmed for release when they are needed the most. Further, in the shadow of the COVID-19 global pandemic, work on the development of efficacious and safe vaccines has hit a faster pace than ever before. Orally administrable vaccines are highly sought for a vast number of globally relevant infectious diseases. Due to its significantly lower levels of digestive pancreatic enzymes, and close ties with immune function, the colon could be a viable solution for oral delivery of vaccines [262,263].

5.1. Novel IBD treatments

Although IBD treatment has broadly remained the same in the last two decades, novel therapies have recently been investigated [264,265]. Amongst these are Janus kinase (JAK) inhibitors which have shown favourable results. JAK inhibitors are tyrosine kinases that function by supressing the action of one or multiple Janus kinase enzymes (e.g., JAK1, JAK2, JAK3, TYK2), thus blocking cyto-

kine signalling. Whilst JAK inhibitors were initially developed for the treatment of rheumatoid arthritis, they are now being explored for the treatment of IBD. This class of small molecules are administered orally and have a short serum half-life and intracellular selectivity, making them well suited for immune-mediated inflammatory conditions [266]. As an example, tofacitinib citrate has been approved for the oral treatment of moderate-to-severe UC, for which it has shown a dose-specific effectiveness and is well tolerated by patients [267]. Similarly, filgotinib and upadacitinib are being evaluated for use in UC and CD and are currently in Phase III clinical trials with promising results.

One drawback of most IBD treatments is that the drugs can be systemically absorbed, increasing the risk of adverse reactions. In order to reduce these effects, several strategies, such as selectively targeting the inflamed area and keeping the formulation localised at its site of action, have been investigated. In this regard, nanomedicines are thought to offer a promising way to deliver molecules with the desired release mechanisms and to overcome formulation problems for drugs with poor aqueous solubility or systemic stability issues [268]. Despite their versatility and promising preclinical *in vivo* results, very little is known about the fate of nanoparticles (NPs) after they reach the site of inflammation, their local drug release mechanism and whether they are degraded by the enzymes present on the mucosa or they are internalised by phagocytes [269]. Thus, in realistic terms they are far from translation into clinics and will not be discussed in detail in this review.

5.2. Chronotherapy: Timing is key

Chronotherapy, with its prefix coming from the Ancient Greek *khrónos* meaning time, describes the technique of working with the body's natural rhythms to optimise a medicine's pharmacokinetics [270,271]. This term can also encompass the targeting of the circadian clock directly for positive therapeutic outcomes (Fig. 12A) [272]. The colon is an ideal site of delivery for chronotherapeutics, because targeted delayed release systems can provide programmed drug release overnight or in the early morning, when many diseases are at their worst [271]. Time-dependent systems may be more adequate given that drug bioavailability is not impacted significantly if drug release occurs more proximal or more distally in the gut.

Asthma is a prime example of a disease affected by circadian rhythm. The risk of asthmatic bronchospasm is increased at night
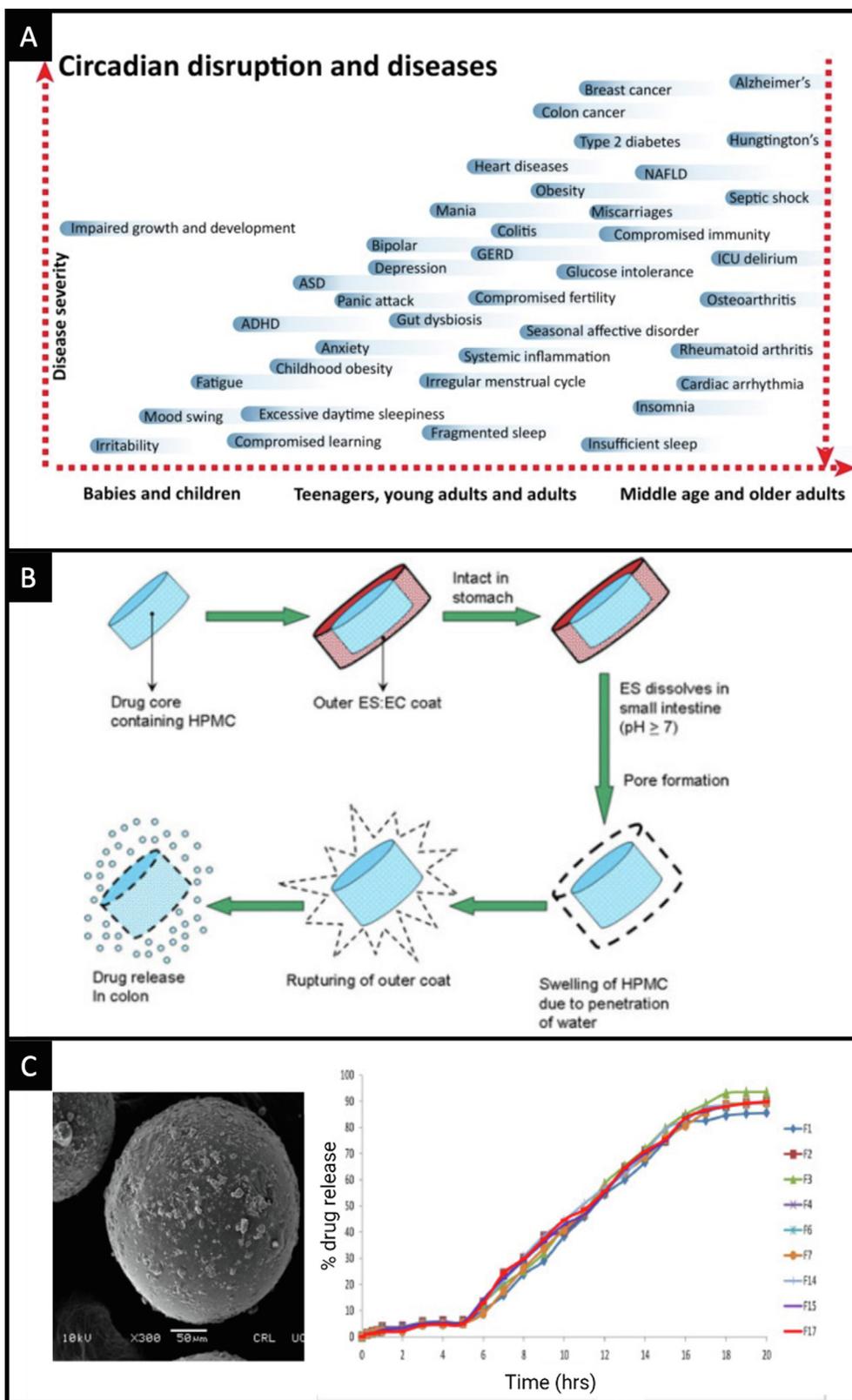
Fig. 12. (A) Diseases linked to circadian disruption and relevant patient populations [272]. (B) A concept for colon-targeted theophylline for nocturnal treatment of asthma [274]; (C) Celecoxib-β-cyclodextrin loaded Eudragit® S 100 microparticles for treatment of early morning rheumatoid arthritis symptoms [275]. Images were reprinted with permission from the original sources.

due to circadian up-regulation of pathways of systemic inflammation and oxidative stress [273]. Colon-targeted medicines allowing nocturnal release of anti-asthma drugs could not just improve patients' symptoms, but also their sleep quality, bringing a great number of physical, psychological and social benefits. Theophylline, a phosphodiesterase inhibiting drug used to treat

moderate to severe asthma, has been formulated into a delayed-release system for this purpose [274]. The 200 mg dose of theophylline was formulated as a tablet, containing the drug in the core together with HPMC (Fig. 12B). This core tablet was coated with a blend of Eudragit® S100 and EC. The formulation was shown to initiate drug release in the colons of rabbits after a lag time of 5 h.

Rheumatoid arthritis is a debilitating condition in which symptoms closely follow a 24-hour rhythm with maximal stiffness, movement difficulty and pain in the early morning [261,275]. To treat this condition, one study proposed celecoxib-β-cyclodextrin loaded Eudragit® S 100 microparticles, releasing the drug at a constant rate in an *in vitro* model at pH 7.4 (simulating colonic conditions), after a lag phase of 5 h at pH 1.2 and 6.8 (Fig. 12C) [275]. Another approach involved filling empty HPMC capsules with mini-tablets containing naproxen, enzyme-sensitive polymers (e.g., guar gum, sodium alginate) and pH-sensitive polymers (e.g., Eudragit® L and Eudragit® S) [276]. *In vitro* drug release was reported to show a lag time of 2 h (at pH 1.2), followed by about 6 h of controlled release at neutral pH [276]. Elsewhere, pro-drugs of β-boswellic acid (an ole gum resin with anti-inflammatory properties) were suggested as potential chronotherapeutics for rheumatoid arthritis treatment [277]. *In vivo* studies in male Wistar rats revealed that the t_{max} of one of the investigated pro-drugs (activated by N-acyl amidases secreted by colonic microorganisms) was about 13 h, which could allow patients to take the medication orally with their evening meal and wake up with pain-relief.

Oncology is a clinical specialty that has somewhat embraced chronotherapy [272,278]. Opportunities for colon-targeted anti-cancer chronotherapeutics are numerous, with many oncogenic signalling pathways linked to circadian rhythms [278,279]. IBD is known to increase the risk of developing GI malignancy, due to chronic inflammation [280]. There is growing evidence that IBD has a bi-directional relationship with circadian regulation. IBD may predispose a patient to circadian disorders, whilst circadian gene disturbance has been seen to increase inflammatory damage in mouse IBD models through cytokine production [281]. For this reason, targeted therapeutics to treat IBD may be more efficacious if they consider circadian rhythms [281]. Sulfasalazine has recently received attention as a novel anticancer agent through its inhibitory actions on the plasma membrane cysteine transporter, xCT [282]. Lately, it was shown that its expression in colon tumours follows circadian patterns, with higher membrane concentrations in the early evening compared to the early morning. When sulfasalazine was administered to colonic tumour-bearing mice in the early evening, tumour volume was shown to be significantly decreased while drug plasma levels increased compared to early morning dosing and to controls [283].

5.3. Microbiome targeted therapeutics

An individual's colonic microbiome is highly unique, with constituents dependent on a myriad of factors including method of birth, age, diet, geographical habitat, hygiene, infection history and past antibiotic courses [265,285-287]. For most individuals, anaerobic bacteria of the *Bacteroidetes* and *Firmicutes* phyla are the core bacterial components of the colon [117,287]. The functions of bacteria in the colon are extensive with implications towards immunity, digestion, vitamin synthesis, neurology and fat storage to name just a few [287-290]. In addition, there is a smaller, less researched population of archaea, viruses, unicellular eukaryotes and fungi [117,290]. The impact on health of these lesser representatives should not however be discounted; the viral and fungal microbiota have been linked to different diseases, including IBD [291-294].

Metabolites produced by microbiota such as SCFAs; branched chain fatty acids (BCFAs); branched chain amino acids (BCAAs); biogenic amines; vitamins; toxins; bile acids and gases, are responsible for the microbiome's effects on health [294-297]. In particular, SCFAs are used as markers of good gut health [120]. SCFAs, primarily acetate, propionate and butyrate, are the end products of fermentation of indigestible carbohydrates by colonic microbes [296]. SCFAs are known to modulate intestinal motility, wound healing, inflammation and intestinal permeability among many other processes important to health [294,297-299]. Acetate and propionate also act as an energy source for peripheral tissues and butyrate for colonocytes [120]. Although the interplay between metabonomics and diseases is hugely complex and highly disease-dependent, SCFA production is a good starting point for the assessment of colonic microbiome health [296,299]. It is also generally accepted that higher diversity of bacterial species within the colonic microbiome is positive for health; diversity-rich communities are less susceptible to pathogenic invasion and have been shown to be protective against diseases such as obesity, IBD and *C. diff* infection [300,301].

5.3.1. Supplementing bugs: Probiotic technologies

Probiotics are live microorganisms that can confer a health benefit when administered at defined doses [302,303]. Probiotics designed for oral administration are notoriously difficult to deliver to the colon. Poor formulation can expose the live microorganisms to the acidic gastric environment and digestive enzymes of the small intestine, leading to loss of viability [120,304]. With strategic formulation development, however, probiotics can successfully be orally delivered for colonic targeting. As an example, vancomycin hydrochloride was formulated into a CODES™ system with a probiotic approach including *Saccharomyces boulardii (S.b.),* intended to remedy antibiotic-induced dysbiosis [305]. Along similar lines, the Phloral® coating has been shown to ameliorate commercial probiotics' survival during *in vitro* GI transit to the colon, and adherence to intestinal epithelial cells [248]. Adherence of probiotic bacteria to the colonic wall is imperative for them to assimilate with commensals and exert beneficial effects. Phloral® has also facilitated effective faecal microbiota transplantation (FMT) therapy to patients with recurrent *C. diff* infection [260]. The 31 patients who received colon-targeted faecal microbiota were shown to have higher cure rates than those who received the same microbiota in a gastric release formulation (80.6% vs. 75%). Both formulations were found to be safe without adverse effects. FMT has been shown to be a highly efficacious treatment method for delivering microbiota directly to the colon for the promotion of higher gut microbial diversity. In addition, FMT is now recommended by medical bodies for patients suffering from recurrent C. *diff* infection who have failed to respond to conventional antibiotic treatment [306].

5.3.2. Drugging the bugs: Microbiome remodelling

Direct remodelling of the gut microbiome has been defined as "a process that can alter, in a deliberate and predetermined fashion, the bacterial composition and/or transcriptome from a given state to another" [307]. As with all drugs delivered to the colon, it is important to recognise the substantial metabolic capacity of microbiota when developing new therapies targeted to the microbiome. Over 150 small molecule drugs are currently known to be susceptible to direct structural transformation by intestinal microorganisms, therefore microbial degradation should be a key investigation for novel microbiome therapeutics [264,308]. Assessment can initially involve *in silico* prediction based on drug structure, for example using ML techniques, followed by experimental validation using *in vitro*, *ex vivo*, or *in vivo* methods [309-312].

Aside from supplementing and promoting multiplication of colonic bacteria, it may be possible to target pathogenic bacteria
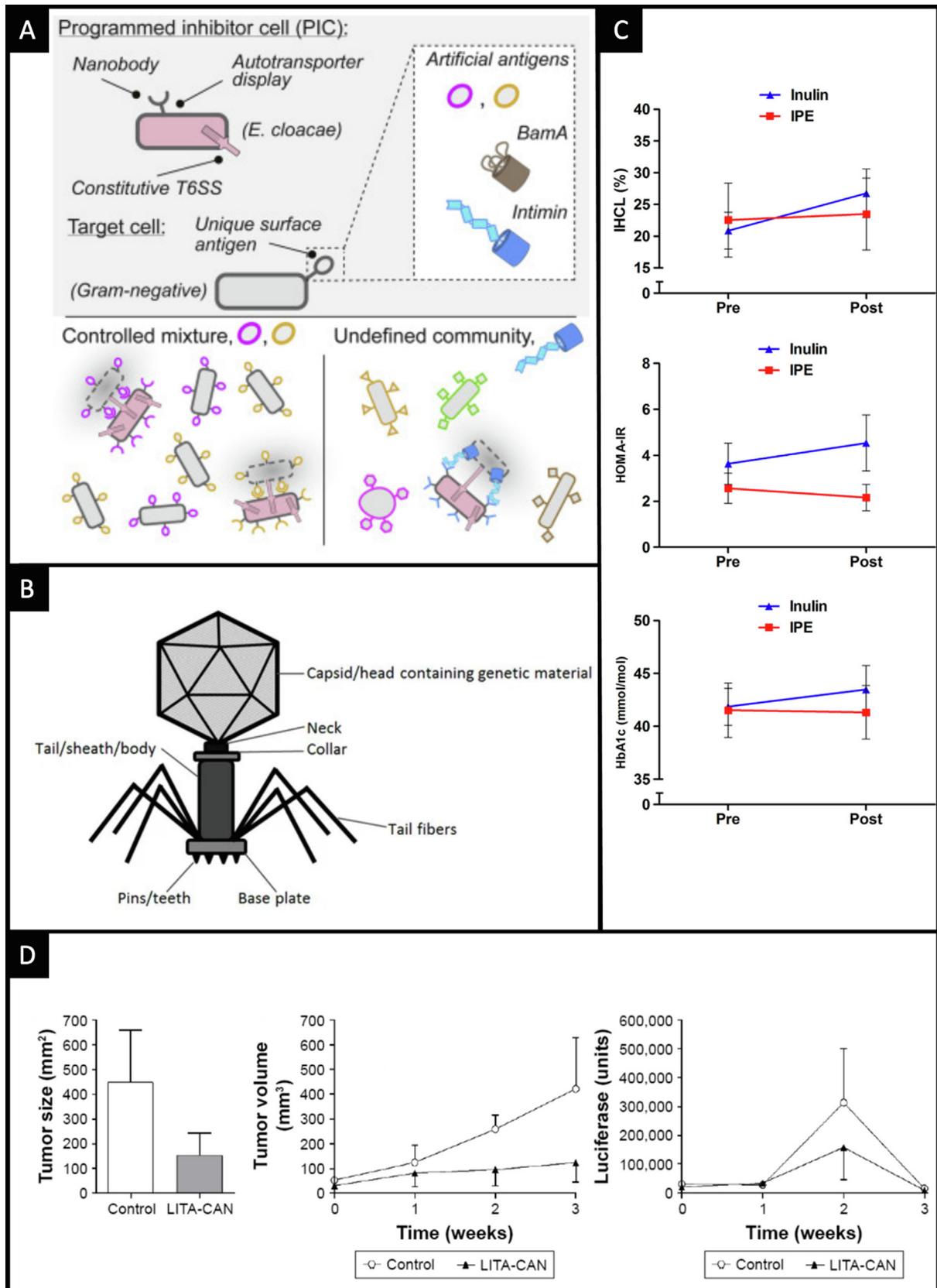
Fig. 13. (A) Inhibitor cells targeted to deplete specific bacteria in a heterogeneous community via programmable cell adhesion [318]. (B) The anatomy of a tailed bacteriophage; bacteriophages are being increasingly investigated for targeted microbiome modulation [320]. (C) Effects in humans of 42 days administration of inulin-propionate ester (IPE) and inulin as a control on intrahepatocellular lipid (IHCL), homeostatic model assessment of insulin resistance (HOMA-IR) and glycosylated haemoglobin (HbA1c) (data expressed as means, n = 9) [321]. (D) The effect of intraperitoneal injection of acetate loaded nanoparticles (NPs) on tumour size, volume and luciferase luminescence of colorectal tumours in mice (n = 8). Images were reprinted with permission from the original sources.

already residing in the colon [312]. The attenuation of pathogenic colonic bacteria with traditional antibiotics is an accomplished practice. A key example is the treatment of *C. diff* infection with vancomycin or fidaxomicin [313]. Traditional antibiotic use, while frequently life-saving and necessary, is however associated with a plethora of side effects and the global concern of antimicrobial resistance [314,315]. It is therefore of paramount importance that alternatives are sought. There is much interest in the discovery of novel narrow-spectrum antibiotics, such as the newly synthesised benzyl thiophene sulphonamide based small molecule with activity against *Campylobacter jejuni* and *Campylobacter coli* [316]. *Campylobacter* is a leading cause of foodborne gastroenteritis worldwide, therefore a new antibiotic with minimal impact on the wider colonic microbiome could prove exceptionally useful [317]. Further, inhibitor cells have been developed that can be programmed to deliver toxic proteins to target cells. By a nanobody-based cell–cell adhesion mechanism, inhibitor cells can recognise and deplete target bacteria of interest in a complex microbial community (Fig. 13) [318]. Orally administered biologics are also receiving attention for precision microbiome remodelling. For example, the company Finch Therapeutics has five biologic compounds in preclinical and clinical development for microbiome-mediated treatment of diseases including IBD and autism spectrum disorder [319].

It may be the case that certain colonic bacteria are neither wholly "good" nor "bad", therefore killing them based on their pathogenic activity could in turn lead to loss of beneficial activity. Release of compounds toxic to human cells, such as lipopolysaccharide (LPS), can also occur when bacterial cells are lysed [322]. With this in mind, inhibition of specific disease-linked enzymes produced by bacteria could be the best clinical decision [312]. The gut enzymes involved in the conversion of dietary trimethylamines to trimethylamine-N-oxide (TMAO) have been shown in several studies to increase the risk of atherothrombotic events [323,324]. By blocking these enzymes, TMAO's thrombotic and atherosclerotic promoting activity is reduced without killing the metabolising bacteria themselves [17,325]. Altering the environment of the colon to favour beneficial microbes could also be a formulation opportunity. For example, it is known that lowering colonic pH boosts butyrate production and butyrogenic species such as *Roseburia* spp. and *F. prausnitzii*, whilst decreasing acetate and propionate synthesis; at higher pH (a transition from pH 5.5 to pH 6.5), the opposite is true with greatly increased levels of *Bacteroides* species [326]. It is also recognised that the alteration of the colonic environment by antibacterial bile salts can have pathogenic effects when in excess, such as gall bladder infection and risk of colorectal cancer [327,328]. A formulation that manages the effects of bile acids on colonic microbes could therefore be advantageous.

5.3.3. Delivering short chain fatty acids (SCFAs)

The direct delivery of SCFAs to the colon could confer health benefits whilst cutting out the complexity of the bacterial "middle-men" [329]. Though colon-targeted SCFA delivery is much less researched than delivery of probiotics and prebiotics, there are a number of publications and clinical trials that give reason for positivity in the strategy. One area of interest is the delivery of butyrate for its anti-cancer properties. In the colon, butyrate partakes in biochemical pathways that promote the apoptosis of cancerous cells and inhibit oncogenic signalling pathways [330,331]. The fact that butyrate is toxic to cancer cells and at the same time beneficial for healthy cells makes it a promising chemotherapeutic agent [330,332]. Despite the strong evidence for its efficacy, translation to the clinic has largely been prevented by the low bioavailability and short half-life in circulation of butyrate [333]. Though a source of butyrate, tributyrin, has been successfully formulated for *in vitro de*livery to the small intestine

[334], there is still some way to go until an approved formulation provides reliable butyrate concentrations in the human colon. With this in mind, intelligent formulations could be the gateway required to bring a new colorectal cancer treatment to market.

The benefit of delivering propionate to the colon for treatment of metabolic diseases has been explored in recent years. As an example, an inulin-propionate ester (IPE) was developed, whereby propionate is covalently bound to inulin, and can be released through microbial hydrolysis in the colon [321,335]. IPE taken daily for 42 days has been indicated to attenuate acetate-mediated increase in intrahepatocellular lipids amongst participants with non-alcoholic fatty liver disease [321]. In an earlier trial including 60 overweight adults, IPE taken for 24 weeks significantly reduced further weight gain, intra-abdominal adipose tissue distribution, intrahepatocellular lipid content, and deterioration in insulin sensitivity [335]. IPE has also been shown to significantly reduce food intake and appetite when taking the supplement for seven consecutive days [336,337].

5.4. Nutrient delivery to the colon

Gut microbiome composition is intrinsically linked to diet [338], where food can be beneficial or harmful to the gut microbiota. Dietary influences have been shown to modulate the colonic mucus; for example, low-fibre diets have been linked to the thinning of the mucus due to bacterial colonisation. The thinning exposes the mucosa, causing an immune response and further damage and inflammation, as seen in UC [39]. Future directions of the field suggest that diet could be leveraged as a tool to modulate gut microbiota. The next phase of this research could involve solid dosage forms that deliver NPs containing amino acid- and nucleotide-based medicines to the colon. These dosage forms in turn may benefit from combination approaches, for example, the continued use of Eudragit® coatings allied to exploitation of bacterial enzymes to ensure release of the NPs.

The role of harnessing synergistic nutrient sensing receptors on colonic endocrine cells has been proposed for the management of obesity. Currently, gastric bypass surgery is the most effective treatment for obesity and type 2 diabetes but it is often limited in availability, irreversible and may cause long term health repercussions [339]. Surgically shunting undigested food to the distal gut triggers hormones release by activating enteroendocrine cells via specific nutrient receptors [340]. Coated with the Phloral® technology, colonic enteroendocrine cells which express nutrient sensing receptors (GRP84 and FFAR4) for medium-chain fatty acids were investigated as a non-surgical intervention for the treatment of obesity [341]. A randomised, double-blind, placebo-controlled, cross-over study in obese adults found that subjects receiving GRP84 and FFAR4 agonists had reduced overall calorific intake and achieved increased postprandial levels of the potent anorectic hormone PYY which aids the regulatory of satiety (Fig. 14).

Another approach involved the exploitation of colonic drug delivery for the management of diabetes. In particular, a sustained release butyrate tablet was coated the Phloral® system [342]. The delivery of butyrate to the colon aids in restoring natural hormone secretion of glucagon-like peptide 1 (GLP-1) in the gut to normalise glucose. Additionally, butyrate has also shown to improve insulin sensitivity, especially in insulin-resistant patients. This concept is potentially applicable to diseases other than diabetes, including non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), and polycystic ovary syndrome (PCOS).

Prebiotics are food sources for gut bacteria and promote the survival and colonisation of the bacteria they feed [294]. It is important to recognise that it is not just the direct metabolising bacteria that benefit from prebiotics, but also the microbiota that feed on the metabolites produced by those bacteria [294].
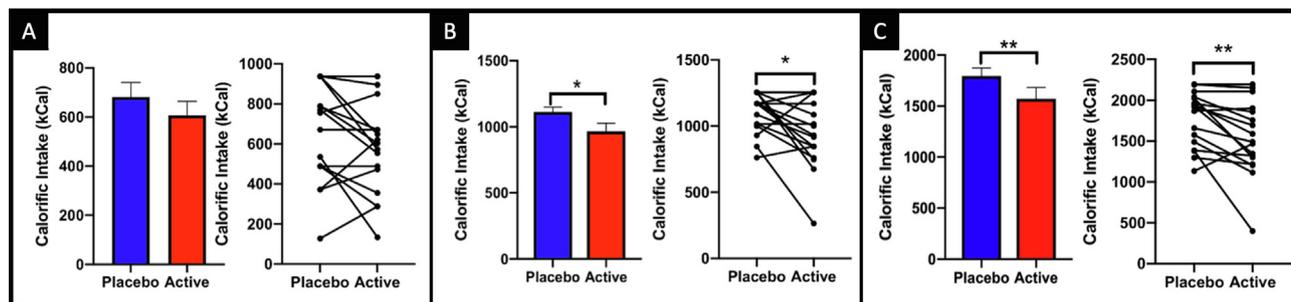
Fig. 14. Colonic delivery of nutrient combinations to obese subjects increases perception of satiety. (A) At breakfast: calorific intake at breakfast was unchanged between the placebo and active treatment arms. (B) During lunch: calorific intake was significantly lower in the active than in the placebo treatment arms (p = 0.019). (C) Total calorific intake was significantly lower in the active than in placebo treatment arms (p = 0.008). Reprinted with permission from [341].

Randomised controlled trials have recently shown that prebiotics could improve the dysbiosis seen in viremic untreated HIV patients [294]. Supplementation with 20 g prebiotics (including short-chain galacto-oligosaccharides, long-chain fructo-oligosaccharides and glutamine) daily for 6 weeks has been seen to increase numbers of *Faecalibacterium* and *Lachnospira,* augment butyrate synthesis and decrease the inflammatory biomarkers CD14 and C-reactive protein (CRP) [343,344]. Although a follow-up trial using a combination of prebiotics and probiotics (known collectively as a synbiotic) did not show a synergistic relationship with anti-retroviral therapy, other long-term benefits could exist [295,345].

5.5. Delivering biopharmaceuticals to the colon

The last two decades have seen an upsurge in the approval and prescription of peptide- and protein-based treatments. In fact, the global top two selling medicines of 2020 (adalimumab and pembrolizumab) were both monoclonal antibodies, highlighting the prevalence of this therapeutic class [346]. Indeed, the global market worth of biopharmaceuticals was estimated at $192.5 billion in 2020 with expected growth to $326.3 billion by 2026 [347]. This growing interest in peptide-based therapeutics is driven by the molecules' often enhanced selectivity, potency and efficacy compared to small molecule drugs, and is supported by peptides' generally good safety profiles [348]. Despite this, almost all peptide therapeutics are formulated for parenteral administration due to their general instability in the upper GI tract. Collectively, fluctuations in pH and enzyme concentrations throughout the GI tract can contribute towards the denaturation of peptides [348]. However, the requirement for injections is less favourable with patients than oral administration, and often necessitates the presence of a healthcare professional, which increases burden on medical systems and the number of appointments patients must attend.

Compared to the proximal gut, the colon may offer an advantageous site for peptide delivery. Protease levels are lower in the colon and longer transit times provide a greater window for absorption of peptides into systemic absorption [17]. Moreover, the colon is more responsive to epithelial absorption enhancers [349]. However, the higher density of microbiota and thicker mucus layer in the colon present challenges not seen in the proximal gut [30]. Research has shown that smaller peptides, such as desmopressin and octreotide, are more stable amongst human faecal microbiota and enzymes than larger peptides, such as insulin and somatostatin [17]. Interestingly, stability within the colonic model was observed to positively correlate with peptide lipophilicity (R^2 = 0.94), potentially because less hydrogen bonding within molecules lowers their binding to degradative enzymes. To facilitate peptide permeation through the outer and inner colonic mucus layers, which is necessary for absorption into circulation, formulation strategies such as mucoadhesion, surface-engineered

NPs, and mucolysis have been investigated [350-353]. At present, there is no accepted best strategy as each method carries its own disadvantages. For example, negatively charged NPs could compromise the colonic immune system through macrophage cytotoxicity [351]. Peptide-loaded NPs have also had disappointingly low translation to clinical trials with scalability issues, low epithelial uptake, uncontrolled release and low particle loading amongst the prominent reasons for early failure [1]. Further, mucolytic agents may expose the colonic epithelium to pathogens and risk systemic microbial invasion. Presence of disease also presents complexity for peptide formulations as intestinal inflammation can lead to depletion of the mucus layer and accumulation of cationic proteins such as transferrin [49,352].

To date, colon-specific delivery of peptide and protein therapeutics has received less commercial attention than small intestinal targeting. Whilst there are a few examples of colonic formulations reaching early clinical trials, such as the oral delivery of infliximab for treatment of IBD, colonic peptide delivery remains a relatively untapped opportunity [353]. By observing the small intestine-targeted therapeutics progressing through clinical trials and gaining market approval, lessons and formulation inspiration could be translated to colonic delivery [1]. For example, the Peptelligence™ system by Enteris Biopharma uses citric acid to protect peptide therapeutics from intestinal peptidases by promoting a protective acidic region around the peptide [354]. This technology has facilitated the delivery of oral calcitonin, achieving superior clinical performance and equal tolerability to an existing nasal formulation in phase 3 trials, for the treatment of postmenopausal osteoporosis [355]. Elsewhere, an oily formulation comprised of a medium fatty acid has been developed for permeation enhancement of intestinally delivered peptides. Known as TPE™, the technology claims to open intestinal tight junctions, and has been applied to the MYCAPSSA™ formulation (Chiasma, United States) for the oral delivery of octreotide for treatment of acromegaly [356]. These cases highlight that intestinal delivery of peptide therapeutics is possible, potentially enabling a paradigm shift from parenteral to oral biologics that will ultimately benefit patient care.

5.6. Colonic vaccine delivery

Orally administered vaccines can be degraded in the upper GI tract by pH-mediated or enzymatic processes. Therefore, targeting vaccines to the colon may circumvent such hostile environments. In this regard, nanotechnologies might offer an interesting potential as vaccine delivery systems [357]. Oral colonic vaccines have been designed to target two difficult to treat disease states: (i) viruses that invade via the mucosal route (e.g. HIV), and (ii) colorectal cancer. Vaccine delivery to the mucosal layers of the colon, rectum and vagina can produce local mucosal protection by potent humoral and cellular immunity through the common mucosal
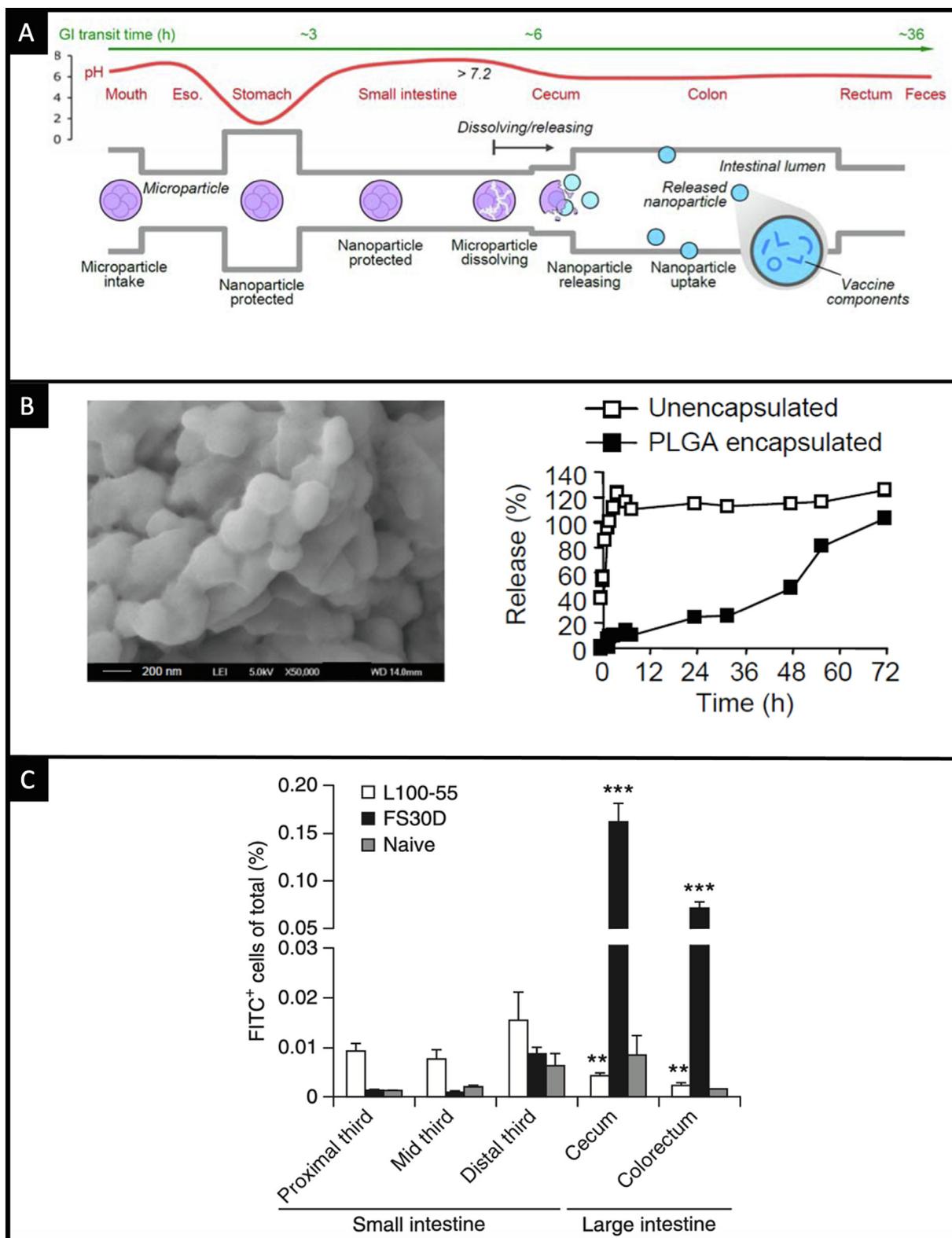
Fig. 15. Colonic targeted vaccine delivery system. (A) Microparticle design and transit for the oral delivery of colonic vaccines; intraluminal pH (red) and GI transit time (green), (B) Image of PLGA/FITC-BSA NPs and release rate of unencapsulated versus BSA-encapsulated PLGA NPs. (C) Gut mucosal uptake of PLGA particles after oral delivery of Eudragit® FS30D/PLGA or L100-55/PLGA. Reprinted with permission from [361] (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

system [358]. An example of this strategy is lyophilised lipid NPs that were loaded with hepatitis B antigen and filled into small capsules, which were subsequently coated with a blend of Eudragit® S

and Eudragit® L [359]. Two to three-fold mucosal immune response was found, compared to a marketed vaccine in rats. Another example is Eudragit® FS30-based microparticles contain-

ing PLGA NPs entrapping a range of *vaccinia*-related antigens and luciferase-expressing DNA plasmids (Fig. 15) [360]. Oral administration resulted in particle uptake by the colonic epithelial and antigen-specific T-cell activation in the colon of female BALB/c mice.

Vaccines for colorectal cancer aim to activate immune effectors (T-cells and antibodies) and infiltrate tumours to prevent reoccurrence or to treat advanced cancers [361]. Approaches can deliver high doses of tumour antigens. However, rapid vaccine degradation may occur due to phagocytosis [362]. As an example, PEGylated graphene oxide nanosheets were loaded with an adenosine diphosphate-dependent glucokinase neoantigen peptide and CpG oligodeoxynucleotide. Extensive characterisation reported good drug loading, size and stability. A single dose of vaccine was significantly able to reduce tumour growth and increase the survival rate of female C57BL/6 mice injected with MC-38 tumour cells [362].

Exosomes have also been recognised as promising vaccine nanoplatforms due to their biocompatible structure and characteristics [363]. Recently, exosomes derived from colonic tumour cells (CT-26) were used to deliver microRNAs, resulting in strong inhibition of tumour growth in BALB/c mouse models [364]. Whilst several colorectal vaccines have showed good results in phase I/II clinical trials, key limitations exist including the time required for sufficient immune response and the immunosuppressive effects [365].

6. Conclusion

The colon is a diverse and dynamic environment with multiple characteristics that make it a unique region of the GI tract. Targeted delivery of drugs to the colon can be favourable for enhancing local therapeutic action (such as for IBD), reaching specific targets (such as microbiota), and improving drug pharmacokinetics. However, traditional formulation strategies aimed at colonic drug delivery can prove unreliable due to their dependence on single trigger mechanisms. Recently, advances in the field have led to the development of superior colon-targeting systems, which exploit multiple characteristics of the colonic environment and exert their actions in parallel to achieve more robust and reliable site-specific drug delivery. With such systems, the colon can be exploited to deliver drug products for the treatment of diseases that extend beyond local disorders of the GI tract, unlocking emerging therapeutic opportunities for the improvement of patient care.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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